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Lipid composition of watermelon seed oil

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Lipid composition of watermelon seed oils with Bulgarian and Greek origin was examined. The oil content was found to be 25.2 and 24.0%, respectively. The main fatty acids in triacylglycerols were linoleic (58.7 – 63.6%), oleic (18.3 – 18.8%) and palmitic (14.0 – 15.0%). Total phospholipid content varied between 3.1 and 6.3%, and the main representatives were phosphatidylcholine (42.2%) in the seeds from Bulgarian origin and phosphatidylserine (26.9%) in those with Greek origin. The main components in the sterol fraction (0.6 – 0.9%) were β-sitosterol (53.5 – 58.9%) and Δ^5-avenasterol (35.9 – 37.4 %). Fatty acid composition of the phospholipids differed from those of triacylglycerols. Saturated fatty acids prevailed in phospholipid fraction, while unsaturated fatty acids – in triacylglycerols. Fatty acid composition of phosphatidylcholine in Bulgarian seed oil was the only exception where unsaturated fatty acids (59.0%) were in a higher amount than saturated (41.0%). Total tocopherol content was established to be 962 – 1010 mg/kg and the main component was γ-tocopherol (92.3 – 97.0%).

Keywords: watermelon seeds, lipid composition, tocopherols, sterols, phospholipids

INTRODUCTION

Watermelon is an annual plant from Cucurbitaceae family. It is widely distributed and cultivated in Africa and the Middle East, but can also be grown in Europe [1 - 3]. The fruit is rich of carbohydrates, carotenoids and lycopene and it is well-known for its high water content [4]. Watermelon is grown in almost every region in Bulgaria, apart from the mountain areas.

Many authors report that watermelon seeds are waste products from the fruit and can be eaten as a roasted snack or used in cooking [1 - 4]. They are abundant in lipids and proteins. The oil content of the seeds is considerably high (27.1 – 57.26%) [1 - 7]. Linoleic acid is the main fatty acid (56.8 – 62.1%), followed by oleic (11.0 – 14.6%), palmitic (10.6 – 15.0%) and stearic acids (8.3 – 16.0%) [2, 6, 8].

The content of unsaponifiable matters ranges from 0.56 to 0.80% and depends on the specific watermelon species [3, 7] and total sterols are 1.12 – 8.1%. The main components in the sterol fraction are $\Delta^{7,22,25}$ – stigmastatrienol (31.6%), $\Delta^{7,25}$ – stigmastadienol (29.7%) and $\Delta^{7,22}$ – stigmastadienol (26.5%) [1, 9].

Total tocopherol content is 131 – 369 mg/kg [3, 7] and according to Raziq *et al*. (2012) [7] the main representative is α-tocopherol (73.8 – 94.1%). On the other hand, Mariod *et al.* (2009) [3] reported that the main tocopherol is γ-tocopherol (97.3%). The information about the total phospholipid content and their individual composition is rather scarce and El-Adawy and Taha (2001) [1] established that total phospholipid content of watermelon seed oil is 0.96%.

Watermelon seed oil can be considered as an alternative source to traditional oil produced in Bulgaria (e.g. sunflower, soybean and rapeseed oil). For now no studies have been conducted on the use of watermelon seeds as an oilseed feedstock for food and industrial purposes in the country.

Therefore, the aim of the present study is to examine the fatty acid composition of watermelon seed oils with different origin (Bulgarian and Greek), as well as the determination of the main biologically active components (phospholipids, sterols and tocopherols).

EXPERIMENTAL

Samples

Two kinds of watermelon seeds with different origin (from Bulgaria and Greece) were used for the analysis.

Isolation of glyceride oil and determination of oil content

The oil was extracted from ground seeds using hexane in a Soxhlet apparatus for 8 h. The solvent was partly removed in a rotary vacuum evaporator, the residue was transferred in a pre-weighed glass vessel and the rest of the solvent was removed under stream of nitrogen to a constant weight to determine the oil content [10].

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Fatty acid composition

Fatty acid composition of triacylglycerols (TAG) was determined by gas chromatography (GC) [11]. Fatty acid methyl esters (FAMEs) were prepared by pre-esterification of the triacylglycerols with sulfuric acid in methanol [12]. Determination of FAMEs was performed on HP 5890 gas chromatograph equipped with a 60 m × 0.25 mm × 25 μm capillary DB-23 column and a flame ionization detector. The column temperature was programmed from 130 °C (hold 1 min), at 6.5 °C/min to 170 °C, at 3 °C/min to 215 °C (hold 9 min), at 40 °C/min to 230 °C (hold 1 min); the injector and detector temperature was 250 °C. Hydrogen was the carrier gas at a flow rate of 0.8 mL/min. Identification was performed by comparison of the retention times with those of a standard mixture of FAME (Supelco, USA 37 comp. FAME mix) subjected to GC under identical experimental conditions.

Determination of phospholipids

Ground seeds were subjected to Folch extraction [13]. Individual phospholipid classes were isolated by two-dimensional thin-layer chromatography (TLC) [14]. Identification was performed by comparing the respective R_f values with those of authentic standards. The quantification was carried out spectrophotometrically at 700 nm after scrapping the phospholipid spot and mineralization of the substance with a mixture of perchloric and sulphuric acid, 1:1 (v/v) [15].

Determination of sterols

Unsaponifiables were determined after saponification of the glyceride oil and extraction with hexane [16]. Quantification of sterols was carried out spectrophotometrically (at 597 nm), after isolation of sterols from other unsaponifiable matter by TLC on silica gel 60 G in the mobile phase diethyl ether: hexane (1:1 (v/v)) [17].

Sterol composition was determined on HP 5890 gas chromatograph equipped with 25 m × 0.25 mm DB-5 capillary column and flame ionization detector. Temperature gradient from 90 °C (hold 3 min) up to 290 °C at a rate of change 15 °C/min and then up to 310 °C a rate of 4 °C/min (hold 10 min); detector temperature – 320 °C; injector temperature – 300 °C and carrier gas was hydrogen. Identification was confirmed by comparison of retention times with those of a standard mixture of sterols [18].

Determination of tocopherols

Determination of tocopherols was carried out by high performance liquid chromatography [19] on 250 mm × 4 mm Nucleosil Si 50-5 column and fluorescence detection at 295 nm excitement and 330 nm emission. The operating conditions were mobile phase of hexane:dioxane, 96:4 (v/v) and flow rate of 1 mL/min.

RESULTS AND DISCUSSION

Lipid composition of watermelon seed oils, as well as the content of biologically active components are shown in Table 1.

Table 1. Lipid composition of watermelon seeds

Compounds	Origin	
	Bulgaria	Greece
Oil content, %	25.2 ± 0.3	24.0 ± 0.2
Phospholipids, %		
- in the oil	6.3 ± 0.1	3.1 ± 0.1
- in the seeds	1.6 ± 0.03	0.8 ± 0.03
Unsaponifiable matter, %		
- in the oil	4.9 ± 0.2	5.1 ± 0.2
- in the seeds	0.3 ± 0.01	0.3 ± 0.01
Sterol content, %		
- in the oil	0.9 ± 0.1	0.6 ± 0.1
- in the seeds	0.06 ± 0.01	0.04 ± 0.01
Tocopherols, mg/kg		
- in the oil	1010 ± 15	962 ± 8
- in the seeds	255 ± 4	231 ± 2

The oil content did not differ in the seeds with Bulgarian and Greek origin (25.2 and 24.0%, respectively). These results were lower than those observed in previous studies where the oil content of watermelon seeds was from 28.5 to 57.2% [1, 2, 4 - 7].

Total phospholipid content of the oil obtained from Bulgarian seeds was two times higher (6.3%) than the content of the Greek seed oil (3.1%). Despite that, these results were much higher than the reported by El-Adawy and Taha (2001) (0.96%) [1].

The content of unsaponifiable matters in the seed oils was 4.9 – 5.1%, which was considerably higher than the data by Raziq *et al.* (2012) [7] and Mariod *et al.* (2009) [3] (0.56 – 0.82%). Total sterol content of the oils was from 0.6 to 0.9%, which was lower than the results reported by El-Adawy and Taha (2001) [1] and Oelschlägel *et al.* (2012) [9] (1.12 – 8.10%).

Total tocopherol content was 1010 and 962 mg/kg, respectively, for the Bulgarian and Greek seed oils, which was much higher than the results from previous studies (131 – 369 mg/kg) [3, 7].

Fatty acid composition of the examined seed oils is presented in Table 2.

The main fatty acid was linoleic acid (63.6 and 58.7 %), followed by oleic (18.8 and 18.3 %) and palmitic (15.0 and 14.0%). The quantity of stearic acid in the oil from Greek seeds was much higher

(6.5%) than in the Bulgarian one (1.3%). This was at the expense of the content of linoleic acid in the same oil. The amount of the other fatty acids ranged from 0.1 to 0.8%. The obtained results were in agreement with these from previous studies [2, 6, 8].

Table 2. Fatty acid composition of triacylglycerols of watermelon seed oils

Fatty acids, %	Origin	
	Bulgaria	Greece
C 10:0 [a]	-*	0.8 ± 0.2
C 12:0	-	0.4 ± 0.1
C 14:0	0.3 ± 0.05	0.4 ± 0.1
C 14:1	-	0.1 ± 0.02
C 15:0	0.1 ± 0.02	-
C 16:0	15.0 ± 0.2	14.0 ± 0.1
C 16:1	0.1 ± 0.05	0.1 ± 0.02
C 17:0	0.1 ± 0.01	0.2 ± 0.02
C 18:0	1.3 ± 0.04	6.5 ± 0.1
C 18:1	18.8 ± 0.2	18.3 ± 0.2
C 18:2	63.6 ± 0.3	58.7 ± 0.2
C 18:3	0.1 ± 0.02	-
C 20:0	0.3 ± 0.02	0.2 ± 0.01
C 20:1	0.2 ± 0.02	0.1 ± 0.01
C 22:0	0.1 ± 0.01	0.2 ± 0.01

*- Not identified

[a]- $C_{10:0}$- Capric acid; $C_{12:0}$- Lauric acid; $C_{14:0}$- Myristic acid; $C_{14:1}$- Myristoleic acid; $C_{15:0}$- Pentadecanoic acid; $C_{16:0}$- Palmitic acid; $C_{16:1}$- Palmitoleic acid; $C_{17:0}$- Margaric acid; $C_{18:0}$- Stearic acid; $C_{18:1}$- Oleic acid; $C_{18:2}$- Linoleic acid; $C_{18:3}$- Linolenic acid; $C_{20:0}$- Arachidic acid; $C_{20:1}$- Eicosenoic acid (gadoleic); $C_{22:0}$- Behenic acid

The content of saturated (SFA), unsaturated (UFA), mono- (MUFA) and polyunsaturated (PUFA) fatty acids of watermelon seed oils is shown in Figure 1.

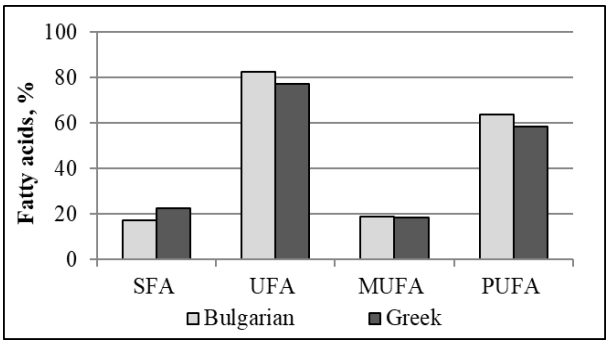

Figure 1. Content of saturated (SFA), unsaturated (UFA), mono- (MUFA) and polyunsaturated (PUFA) fatty acids of watermelon seed oils

UFA predominated in both oils (77.3 – 82.8%) and the share of the PUFA was bigger (58.7 – 63.7%) than MUFA (18.6 – 19.1%). The content of SFA ranged from 17.2 to 22.7% and it was higher in the oil from Greek seeds.

The individual sterol composition of the examined seed oils is shown in Table 3.

Table 3. Individual sterol composition of watermelon seed oils

Sterols, %	Origin	
	Bulgaria	Greece
Cholesterol	0.7 ± 0.1	0.4 ± 0.05
Campesterol	0.6 ± 0.1	0.5 ± 0.06
Δ^7 – Campesterol	2.1 ± 0.1	1.6 ± 0.1
Stigmasterol	2.5 ± 0.05	0.7 ± 0.05
β – Sitosterol	53.5 ± 0.5	58.9 ± 0.4
Δ^5 – Avenasterol	37.4 ± 0.4	35.9 ± 0.2
Δ^7 – Stigmasterol	2.5 ± 0.05	1.6 ± 0.1
Δ^7 – Avenasterol	0.7 ± 0.02	0.4 ± 0.02

The main component in both oils was β – sitosterol (53.5 – 58.9%), followed by Δ^5 – avenasterol (35.9 – 37.4%). The other sterols were presented in quantities from 0.4 to 2.5%. The sterol composition of the oil from Bulgarian seeds did not differ from that of Greek origin. On the other hand, the results were completely different from these reported by Oelschlägel *et al. (*2012) [9], where the main sterol was $\Delta^{7,22,25}$ – stigmastatrienol (31.6%), followed by $\Delta^{7,25}$ – stigmastadienol (29.7%) and $\Delta^{7,22}$ – stigmastadienol (26.5%).

Individual tocopherol composition of the watermelon seed oils is presented in Table 4.

Table 4. Tocopherol composition of watermelon seed oils

Tocopherols, %	Origin	
	Bulgaria	Greece
α-Tocopherol	5.4 ± 0.2	2.0 ± 0.1
γ- Tocopherol	92.3 ± 0.6	97.0 ± 0.5
δ- Tocopherol	2.3 ± 0.1	1.0 ± 0.05

γ-Tocopherol (92.3 – 97.0%) predominated in the tocopherol fraction of the oils and considerable small amounts of α-tocopherol (5.4 – 2.0%) and δ-tocopherol (2.3 – 1.0%) were detected. The results were in agreement with these by Mariod *et al.* (2009) [3], who reported that γ-tocopherol was also the main tocopherol in watermelon seed oil (97.3%), but were different from the data by Raziq *et al.* (2012) [7], who established that α-tocopherol predominated (73.8 – 94.1%).

Phospholipid composition of the examined oils is shown in Table 5.

Table 5. Phospholipid composition of watermelon seeds oils

Phospholipids, %	Origin	
	Bulgaria	Greece
Phosphatidylcholine	42.2 ± 1.1	22.8 ± 0.1
Phosphatidylinositol	22.0 ± 0.9	16.2 ± 0.4
Phosphatidylethanolamine	10.6 ± 0.5	17.6 ± 0.1
Phosphatidic acids	16.8 ± 0.3	16.5 ± 0.1
Phosphatidylserine	8.4 ± 0.2	26.9 ± 0.4

As could be seen, phospholipid composition of the oils differed a lot. While the main component of the oil from Bulgarian seeds was phosphatidylcholine (42.2%), followed by phosphatidylinositol (22.0%), all phospholipid classes in the oil from Greek seeds were present in almost similar amounts – from 16.2% (phosphatidylinositol) to 26.9% (phosphatidylserine). The content of phosphatidylethanolamine and phosphatidylserine was higher in the Greek oil (17.6 and 26.9%, respectively), while the amount of phosphatidic acids was almost the same in both oils (16.5 – 16.8%). These differences could also be explained by the agro-meteorological conditions and geographic regions where the seeds had been grown.

The major fatty acids of the main phospholipid classes are presented in Figure 2.

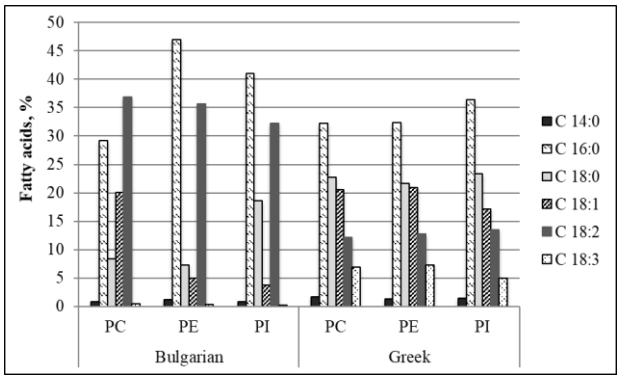

Figure 2. Fatty acid composition of the main phospholipids in watermelon seed oils.
$C_{14:0}$- Myristic acid; $C_{16:0}$- Palmitic acid; $C_{18:0}$- Stearic acid; $C_{18:1}$- Oleic acid; $C_{18:2}$- Linoleic acid; $C_{18:3}$- Linolenic acid; PC- Phosphatidylcholine; PE- Phosphatidylethanolamine; PI- Phosphatidylinositol

The fatty acid composition of the main phospholipid classes (phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol) of watermelon seed oils was examined for the first time. It was observed that the fatty acid composition of the major phospholipid classes of the examined seed oils differed a lot.

Palmitic acid (32.3 – 47.0%) was the main fatty acid in the phospholipids from both examined watermelon seed oils, except in phosphatidylcholine in the Bulgarian oilseed (29.2%). The main fatty acid in the latter was linoleic acid (36.9%). The content of stearic acid in the phospholipids from seeds with Greek origin ranged from 21.6 to 23.3% and it was higher than those of the same fatty acid in the phospholipids from Bulgarian seed oil (7.3 – 18.6%). On the other hand, the amount of linoleic fatty acid in the main phospholipids of the oil from Bulgaria (32.3 – 36.9%) was much higher than these with Greek origin (12.1 – 13.5%). The content of linolenic acid was lower in the phospholipids from Bulgarian seed oil (0.2 – 0.5%), while their quantity in the phospholipids from Greek seed oil ranged from 5.0 (in phosphatidylinositol) to 7.3% (in phosphatidylethanolamine).

The content of saturated (SFA), mono- (MUFA) and polyunsaturated (PUFA) fatty acids of the main phospholipid classes of watermelon seed oils is shown in Figure 3.

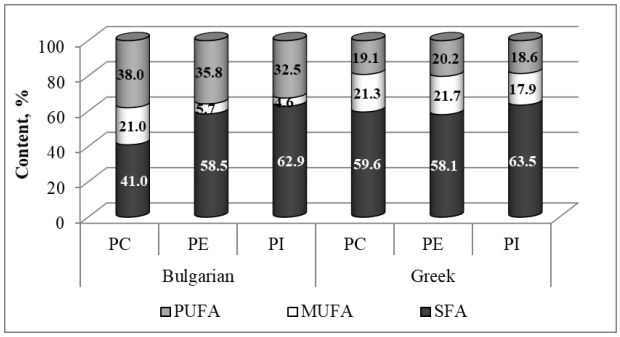

Figure 3. Content of saturated (SFA), mono- (MUFA) and polyunsaturated (PUFA) fatty acids of the main phospholipid classes of watermelon seed oils.
PC- Phosphatidylcholine; PE- Phosphatidylethanolamine; PI- Phosphatidylinositol

The contents of SFA, MUFA and PUFA in the main phospholipids of the watermelon seed oil from Bulgaria differed from those from Greece. SFA predominated in all phospholipids of the examined oils (58.1 – 63.5%) except in the phosphatidylcholine from Bulgarian oil (41.0%), where the main fatty acids were UFA (59.0%). PUFA were found in much higher amount in the phospholipids of Bulgarian seed oil (32.5 – 38%) than in the Greek one (18.6 – 20.2%). On the other hand, the content of MUFA was much lower in phosphatidylethanolamine and phosphatidylinositol of the Bulgarian oil (5.7 and 4.6%, respectively). Overall, fatty acid composition of the main phospholipids of the examined oils was completely different from those of triacylglycerols. While the

SFA predominated in the first ones, the content of UFA was higher in the triacylglycerols.

CONCLUSIONS

The examined watermelon seeds are relatively rich in glyceride oil, which contains a high amount of biologically active substances. There are some differences in the total sterol content and individual phospholipid composition in the watermelon seed oils due to their different origin, climatic and agro-meteorological conditions. In conclusion, due to the lipid composition of the examined watermelon seed oils they can be successfully used as an alternative source of glyceride oil and potentially used in the food industry and cosmetics.

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